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<b>(21) International Application Number:</b> PCT/AU95/00140 <b>(22) International Filing Date:</b> 16 March 1995 (16.03.95) <b>(30) Priority Data:</b> PM 4465 16 March 1994 (16.03.94) AU <b>(71) Applicants (for all designated States except US):</b> THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH [AU/AU]; 300 Herston Road, Herston, QLD 4029 (AU). COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION [AU/AU]; Limestone Avenue, Campbell, ACT 2601 (AU). THE UNIVERSITY OF MELBOURNE [AU/AU]; Royal Parade, Parkville, VIC 3052 (AU). THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH [AU/AU]; Royal Melbourne Hospital, Royal Parade, Parkville, VIC 3052 (AU). BIOTECH AUSTRALIA PTY. LIMITED [AU/AU]; 28 Barcoo Street, Roseville, NSW 2069 (AU). CSL LIMITED [AU/AU]; 45 Poplar Road, Parkville, VIC 3052 (AU). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MOSS, Denis, James [AU/AU]; 29 Mitchell Street, Arana Hills, QLD 4054 (AU). BURROWS, Scott, Renton [AU/AU]; 4 Thumon Court,		Bald Hills, QLD 4036 (AU). KHANNA, Rajiv [IN/AU]; 59 Aberleigh Road, Herston, QLD 4006 (AU). KERR, Beverley, Mavis [AU/AU]; 5 Walmer Court, Carindale, QLD 4152 (AU). BURROWS, Jacqueline, Margaret [AU/AU]; 4 Thumon Court, Bald Hills, QLD 4036 (AU). SUHRBIER, Andreas [DE/AU]; 9 Mark Street, Newmarket, QLD 4051 (AU). <b>(74) Agent:</b> F.B. RICE & CO.; 28A Montague Street, Balmain, NSW 2041 (AU). <b>(81) Designated States:</b> AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).  <b>Published</b> With international search report.
<b>(54) Title:</b> CYTOTOXIC T-CELL EPITOPES  <b>(57) Abstract</b>  The present invention provides cytotoxic Epstein-Barr virus T-cell epitopes. The epitopes are selected from the group consisting of QAKWRLQTL, RYSIFFDY, HLAAQGMAY, YPLHEQHGM, SVRDRLARL, AVLLHEESM, VSFIEFVGW, FRKAQIQGL, PYLPWLAAL, TVFYNIPPMPL, PGDQLPGFSDGRACPV, VEITPYKPTW, and variants thereof. In addition, the present invention provides compositions including these epitopes for use in inducing CTL's in a subject.		

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**CYTOTOXIC T-CELL EPITOPES****Field of the Invention**

The present invention relates to cytotoxic T-cell (CTL) epitopes within Epstein-Barr virus. The present invention also relates to the use of the epitopes in subunit vaccines.

**Background of the Invention**

Epstein-Barr virus (EBV) is a herpes virus that infects approximately 80% of individuals in Western societies. Following primary infection, a life long latent EBV infection of B cells is established. When primary infection is delayed until adolescence, which occurs in 10-20% of individuals in Western societies, there is an approximately 50% chance of developing infectious mononucleosis.

EBV has the very useful property of being able to "immortalise" or transform human B cells. These transformed B cells (referred to as LCLs) have the potential for essentially unlimited growth in the laboratory. There are two methods by which these LCLs can be established. Firstly, they may be established by the use of a common strain of EBV, referred to as B95.8. The LCL that is established is infected with this strain of virus. Secondly, LCLs may be generated using the latently infected B cells, present in all EBV immune individuals as a source of transforming virus. In this case, the LCL that emerges is transformed with the strain of EBV naturally present in any given EBV immune individual (referred to as spontaneous LCL).

There are two EBV types, A and B. The A type appears to predominate in the majority of lymphoid infections of healthy seropositive individuals. In such individuals, latently infected B cells appear to be controlled by CD8+ cytotoxic T cells (CTL) specific for the latent antigens, which include the EBV nuclear antigens (EBNAs) 2-6 and the latent membrane antigens

(LMP) 1-3 (Moss, D.J. *et al.* 1992). Recent developments suggests that CD4+ CTL may also play a part in controlling this infection. These CTL are known to recognise short peptide epitopes derived from antigenic determinants in association with MHC class I molecules on the surface of an appropriate antigen presenting cell. LCLs displaying HLA class I and II alleles and presenting epitopes within EBV latent antigens are frequently used as a target cell for defining the specificity of CTL clones.

As whole virus or recombinant vaccines based on full length latent proteins are considered potentially oncogenic, an EBV vaccine based on CTL epitopes derived from the latent antigens is currently being developed (Moss, D.J. *et al* 1993). Khanna *et al*, (1992) have previously described several CTL epitopes.

#### Summary of the Invention

In a first aspect the present invention consists in cytotoxic T-cell epitopes from Epstein-Barr virus.

More specifically, there is provided twelve cytotoxic T-cell epitopes from the Epstein-Barr virus latent antigens having the amino acid sequences QAKWRLQTL, RYSIFFDY, HLAAQGMAY, YPLHEQHGM (YPLHKOHGM, YRLHEOHGM, YPLHEORGM) SVRDRLARL, AVLLHEESM (TVLLHEESM and TALLHEESM), VSFIEFVGW, FRKAQIQGL, PYLFWLAAI, TVFYNIPPMPL, PGDQLPGFSDGRACP $\bar{V}$  and VEITPYKPTW. In addition, the underlined amino acid sequences in brackets are variants of the aforementioned sequence and have been sequenced from geographically different isolates of Epstein-Barr virus. It has not as yet been established whether these variants are CTL epitopes.

In a second aspect the present invention consists in a composition for use in inducing CTL's in a subject, the composition comprising at least one cytotoxic Epstein-Barr virus T-cell epitope according to the first aspect of the present invention in admixture with at least one

pharmaceutical acceptable adjuvant, carrier, diluent or excipient.

In a third aspect the present invention consists in a method of preparing a composition for use in inducing CTL's in a subject, the method comprising admixing at least one cytotoxic Epstein-Barr virus T-cell according to the first aspect of the present invention with at least one pharmaceutical acceptable adjuvant, carrier, diluent or excipient.

As used herein the term "subject" is intended to cover human and non-human animals.

#### Brief Description of the Drawings

Figure 1. Screening overlapping peptides of EBNA4 on PHA blast cells for reactivity

Figure 2. Reactivity of clone CS30 against a panel of anti- $\mu$  B cell blasts infected with recombinant vaccinia virus encoding the EBV latent antigens.

Figure 3. Minimalisation experiment to define the active epitope sequence within VTAVLLHEESMQGVQVHGSM. This has enabled the definition of the minimal epitope as AVLLHEESM.

#### Best Method of Carrying out the Invention

The following examples illustrate the localisation of twelve new CTL epitopes within the EBV latent antigens using an overlapping peptide net spanning the relevant EBV antigen. The peptides were synthesised using the sequence of the B95.8 strain of EBV (Baer *et al* 1990). In addition, field isolates from different geographic locations were sequenced at the site of CTL epitopes YLPHEQHGM and AVLLHEESM and variants of these epitopes defined.

#### Abbreviations

CTL cytotoxic T-cell lymphocyte  
E effector  
EBV Epstein-Barr virus

HLA human leucocyte antigen  
IL-2 Interleukin-2  
LCL lymphoblastoid cell line  
PBMC peripheral blood mononuclear cells  
5 PHA phytohaemagglutinin  
rIL-2 recombinant Interleukin-2  
T target  
TCM T cell medium  
U/ml units per millilitre

10 Example 1: Basic Culture Media for the Growth of Cells

The medium was RPMI 1640 (Commonwealth Serum Laboratories, Victoria) supplemented with 10% heat-inactivated foetal calf serum, penicillin (100IU/ml) and streptomycin (100mg/ml). Where indicated, this medium was  
15 supplemented with purified recombinant interleukin-2 (rIL-2) (50U/ml; Hoffman La-Roche) and 30% (v/v) heat-inactivated (56°C, 30 minutes) supernatant from the MLA 144 T-cell line. This supplemented medium was called T-cell medium (TCM) and was used in the culture of  
20 phytohaemagglutinin blast cells (PHA blasts) and for the isolation and growth of cytotoxic T-cells (CTL).

Example 2: Preparation of Mononuclear Leucocytes and Generation of CTL

Peripheral blood mononuclear cells (PBMC) were  
25 separated (400g or 1500rpm, 20 minutes) from heparinized (10U/ml) blood on Ficoll-Paque (Pharmacia, Uppsala, Sweden). The separated PBMCs were washed once in basic media and subsequently used either to stimulate CTL, PHA blasts or to establish EBV transformed cell lines.

30 Example 3: HLA Typing

HLA typing of donors was performed by serology.

Example 4: Establishment of Cell Lines

4.1 Establishment of EBV Transformed LCLs by  
addition of exogenous virus: EBV transformed LCLs were  
35 established from PBMCs as follows. The EBV virus stock (Table 1: specific Type and isolate), stored in liquid

nitrogen is selected and rapidly thawed at 37°C. Half a millilitre of virus stock ( $10^5$  transformation units) is added directly into the PBMC cell pellet ( $1-4 \times 10^6$  cells) and incubated for one hour at 37°C. The cells are then washed twice with media at 1000rpm for five minutes. The cells were then made up in media with PHA (Sigma PHA-P) at 2µg/ml and dispensed into a 24 well plate at  $2 \times 10^6$  cells/well. Clumps of cells, representing proliferating LCLs, occur within 1-3 weeks after which the cells were transferred into flasks.

4.2 Establishment of spontaneous LCLs by addition as a means of sampling field isolates of the virus: PBMCs from healthy EBV immune individuals from Australia (Brisbane), from Papua New Guinea (Goroka and Madang) or from Kenya were seeded by doubling dilution from  $2 \times 10^6$  to  $1.25 \times 10^5$  cells per 0.2ml microtitre plate well in culture medium containing 0.1 mg/ml cyclosporin A (Sandoz Ltd., Basle, Switzerland). The cyclosporin A was maintained in the culture medium in regular refeedings for up to 8 weeks. Wells in which proliferation became apparent were subcultured and expanded at 37°C. This method was used to generate field isolates of the virus.

4.3 Establishment of PHA Blast Cell Lines: PBMC ( $2 \times 10^6$  cells/24 well) were stimulated with PHA-P (2µg/ml, final concentration) (Sigma) and after three days, TCM was added. Cultures were expanded into flasks and maintained for up to six weeks with bi-weekly replacement of TCM (without further addition of PHA).

4.4 Generation of anti-µ B cell blasts: PBMCs were separated on Ficoll-Paque (Pharmacia, Uppsala, Sweden) and depleted of T cells using E-rosetting. The enriched B lymphocytes were cultured in growth medium containing anti-IgM (µ-chain specific) coupled to acrylamide beads (Bio-Rad, California, USA), recombinant human interleukin-4 (rIL-4; 50U/ml; Genzyme, USA) and highly

purified recombinant human IL-2 from E.coli (rIL-2; 20-40U/ml) (17,18). After 48-72 hr, B cell blasts were suspended in growth medium supplemented with rIL-2 (20-40U/ml) (14). The B cells continue to divide 2-3  
5 times/week for 3 weeks in the presence of rIL-2. These cells are referred to as anti- $\mu$  B cell blasts.

#### Example 5: Synthesis of Peptides

5.1 Production: Peptides (purchased from Chiron Mimetopes, Melbourne) were synthesised using the pin  
10 technology in duplicate on polyethylene pins and cleaved from the pins. A C-terminal glycine ester link was used in the preparation of peptides with acid C-termini (Valerio, R.M. et al 1991).

5.2 Toxicity and Solubilization: Freeze dried  
15 peptides were dissolved first in 20 $\mu$ l DMSO and then 0.6ml distilled water to give a concentration of 2mM. They were stored at 20°C prior to use. Peptides were diluted in RPMI 1640 for use. Toxicity testing of all the peptides was performed prior to screening by adding peptide at a  
20 final concentration of 100 $\mu$ M to 10<sup>4</sup> <sup>51</sup>Cr labelled PHA blasts in 200 $\mu$ l, in the absence of any effectors.

#### Example 6: Generation of CTLs:

6.1 Generation of polyclonal CTLs Polyclonal CTL  
25 effectors were generated by stimulating PBMCs from healthy seropositive donors with autologous A-type EBV transformed lymphoblastoid cell lines (LCLs) on days 0 and 7. No IL-2 was added to these cultures, as its presence favoured the expansion of non-specific T-cells (data not shown).

6.2 Agar Cloning of T-Cells T-cell clones from  
30 individual donors were generated as follows. PBMC's were isolated and suspended in medium at a concentration of 2 x 10<sup>6</sup> cells in 24 well plates (Costar, Cambridge, Mass). LCL's from the same donor were irradiated at 8,000 rad and added to each of these wells at either 10<sup>5</sup> or 10<sup>4</sup>  
35 cells/well. After three days, cells were dispersed and



seeded in 3.5cm diameter culture dishes in 0.35 agarose (Seaplaque, FMC Corp., Rockland, ME) containing RPMI 1640, 20% 2 x RPMI 1640, 20% FCS, 16% MLA supernatant and 50U/ml rIL-2. Colonies appear within the agar after five days.

5 These are identified under the inverted microscope (x25 magnification) as clusters or chains or discrete cells. These colonies are harvested under the microscope in a laminar flow cabinet by suction into a Gilson pipette. harvested colonies are dispersed into T-cell growth medium (RPMI 1640, 20% FCS, 30% MLA supernatant and 20U/ml rIL-2) and transferred to a 96 well microtitre tray containing irradiated LCL's from the same donor ( $10^4$  cells/well). These colonies continue to be expanded and are stored in liquid nitrogen (approximately  $5 \times 10^6$  cells/ampoule).

15 Example 7: Vaccinia virus recombinants:

Recombinant vaccinia constructs for different EBV latent antigens have been previously described (Khanna et al 1992). All EBV sequences were derived from the B95.8 strain of virus. All constructs had the potential to encode the relevant full length EBV protein.

20 Example 8: Chromium Release Assay:

8.1 Screening CTL clones for reactivity against recombinant vaccinia encoding EBV latent antigens:

25 Anti- $\mu$  B cell blasts were infected with recombinant vaccinia viruses at a multiplicity of infection of 10:1 for 1 hour at 37°C. After 14-16 hours, cells were washed with basic culture medium and incubated with  $^{51}\text{Cr}$  for 90 minutes, washed three times and used as targets in a standard 5 hour  $^{51}\text{Cr}$  release assay as described below.

30 8.2 Peptide Screen: A standard five to six hour chromium release assay was performed on either polyclonal T-cell effectors or T-cell clones, to assess specificity for the peptide epitope. Briefly, washed  $^{51}\text{Cr}$  (Amersham International, England) labelled (60 minutes, 37°C) target cells (autologous PHA blasts) were added ( $10^4$  cells/well in 40 $\mu$ l) to 10 $\mu$ l of peptide (final concentration 100 $\mu$ M) in

a U-well 96 well plate (Nunc, Denmark). After a 30 minute incubation at 37°C, between  $10^4$  and  $50 \times 10^4$  effector T-cells (cloned or bulk CTLs), in triplicate, were then added per well in 150µl, to obtain a final effector to target ratio [E:T] of 50-1:

1. Two controls were added; (i) media and target (background release) and (ii) targets (total release) for addition of 100µl 0.5SDS after the five hour incubation. The plate was then centrifuged at 500 rpm for five minutes and incubated at 37°C for five hours. Following centrifugation at 1000 rpm for five minutes, 100µl supernatants were removed for gamma counting. Results are expressed as % chromium release calculated as mean counts of experimental wells - mean counts of control (background) wells/by total available counts determined by SDS solubilisation - mean counts of control (background) wells.

**Example 9: PCR sequencing of EBC isolated from Australia, Papua New Guinea and Kenya**

The polymerase chain reaction (PCR) was used to amplify specific EBV DNA sequences in the CTL epitope regions YPLHEQHGM and AVLLHEESM. The purified DNA used in the PCR was from spontaneous LCLs from health individuals from Australia, Papua New Guinea and Kenya. Each DNA sample was subjected to two different PCR reactions. One using primers flanking the known YPLHEQHGM region;

E3YPL5 (GAC GAG ACA GCT ACC AG)

E3YPL3 (GAG ATA CAG GGG GCA AG)

and one using primers flanking the known AVLLHEESM epitope region;

E41VT5 (TTG TTG AGG ATG ACG ACG)

E41VT3 (CAG TAG GGT TGC CAT AAC)

Each PCR reaction consisted of 1 x PCR buffer (Boehringer), 0.2mM dNTPs, the purified DNA, the respective primers and 1.5U of Taq polymerase and then

subjected to denaturation at 95°C for 5 minutes followed by 94°C for 15 seconds, 55°C for 30 seconds, 72°C for 15 seconds (35 cycles) using a Perkin Elmer 9600 PCR machine. The PCR products were then purified for sequencing using QIAquick spin columns from Qiagen and sequenced in both directions using the respective primers from the PCR reactions. A PRISM<sup>TM</sup> Ready Reaction DyeDeoxy<sup>TM</sup> Terminator Cycle sequencing kit was used to set up the sequencing reaction. Reaction samples were then run on an ABI Cycle Sequencer.

#### Example 10: Identification of CTL Epitopes Within EBV Latent antigens

By way of example of the epitopes described in Table I, the following methodologies were used in the definition of the CTL epitope AVLLHEESM. The methods used to identify other epitopes were identical. On day 10-11, polyclonal CTLs were used to screen for reactivity against overlapping peptides from EBNA2, EBNA3, EBNA4 and LMP2A on autologous PHA blasts. As seen in Figure 1, after screening all of the peptides from EBNA4 with polyclonal CTLs from donor CS, a single peptide, referred to as peptide 52 (VTAVLLHEESMQVQVHGSM) showed strong reactivity in a <sup>51</sup>Cr-release assay.

To confirm that this sequence was an active CTL epitope, CTL clones from donor CS were established and screened for reactivity against recombinant vaccinia-infected targets (Figure 2). As seen in this Figure, clone CS30 recognised B cell blasts infected with recombinant vaccinia encoding EBNA4.

To minimise the epitope within this 20 mer sequence, overlapping 9 mer peptides were synthesized, and as can be seen AVLLHEESM is the confirmed minimal epitope within the 20 mer peptide. The overlapping peptides from EBNA4 used to demonstrate this minimal overlapping sequence are listed on Figure 3. A/B EBV type specificity and HLA restriction was determined using standard

protocols and demonstrated that the epitope was restricted through HLA B35 and was type A specific (Khanna et al, 1992).

As already mentioned the overlapping peptides were based on the EBV sequence of the B95.8 virus. It was important to determine if field isolates of the virus from different geographical locations also included this sequence. Using PCR sequencing of the EBV sequence present in spontaneous cell lines derived from healthy individuals from Australia (Brisbane), from Papua New Guinea (Goroka and Madang) and from Kenya identified two variants. Two variants of the B95.8 sequence were defined, TVLLHEESM and TALLHEESM (Table 2).

Example 11: Subunit Vaccines

11.1 Vaccine Formulation: Development of new vaccines against a variety of diseases, particularly viral infections, where CD8+ cytotoxic T-cells (CTL) play an important protective role has been hampered by the inability of conventional vaccine formulations to induce protective CTL. Although CTL are readily induced when attenuated viruses are used, in many cases attenuation is difficult, inappropriate and/or unreliable. Conventional killed virus or recombinant protein formulations do not normally gain access to the cytoplasm of antigen presenting cells (APC) and are thus not appropriately processed and presented on class I MHC. A variety of vaccination strategies have been developed to deliver antigen to the cytoplasm of APCs (ie. immunostimulatory complexes [ISCOMs], DNA, fusogenic proteolysosomes and virus like particles). Such approaches often involve complex formulations which can be difficult to standardise, can result in unstable products and/or may only work for antigens with specific characteristics. An alternative strategy has been to use synthetic peptide CD8+ CTL epitopes as immunogens. This approach has several general advantages; peptides are stable, well

defined, easy to manufacture, no infectious material is required for manufacture and the use of potentially pathogenic recombinant proteins can be avoided.

CTL epitopes formulated with Incomplete Freund's  
5 adjuvant (IFA) usually in the presence of a CD4+ helper epitope, have been used to induce CTL in a number of animal systems. Unfortunately, IFA is extremely unlikely, due to its toxicity, to ever be licensed for use in humans. Scalzo, T. et. al. (in preparation) have examined  
10 several adjuvants currently approved, or close to approval, for use in humans to ascertain which would be able to induce protective CTL with a synthetic peptide immunogen. Protection was assessed using the Balb/c murine cytomegalovirus (MCMV) model, in which the  
15 predominant protective response has been shown to be due to CD8+ CTL directed against the epitope YPHFMPTNL, derived from the immediate early antigen 1 (IE-1). The presence of active CTL was confirmed using *in vitro* CTL assays. Scalzo, T. et. al. found that only one  
20 formulation, Montanide ISA 720/ tetanus toxoid/ peptide efficiently induced protective CTL.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments  
25 without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Table 1

CTL peptide epitopes of Epstein-Barr virus within the latent antigens of the virus

CTL EPITOPE SEQUENCE	EPITOPE INCLUDED IN:	HLA RESTRICTION	A/B SPECIFICITY
QAKWRLQTL	EBNA3	B8	A
RYSIFFDY	EBNA3	A24	A
HLAAQGMAY	EBNA3	UNDEFINED	A
YPLHEQHGM *YPLHKQHGM *YRLHEQHGM *YPLHEQRGB	EBNA3	B35.(3)	A
SVRDLRL	EBNA3	A2	A/B
AVLLHEESM #TVLLHEESM #TALLHEESM	EBNA4	B35	A
VSFIEFVGW	EBNA4	B57	A/B
FRKAQIQGL	EBNA6	B57	A
PYLFWLAAI	LMP2A	A23	A/B
TVFYNIIPMPL	EBNA2	HLA DR/DQ	A
VEITPYKPTW	EBNA4	B44	A
PGDQLPGFSDGRACP	EBNA3	A29	A

- 5     \* sequence variants of YPLHEQHGM  
      # sequence variants of AVLLHEESM

Existence and Use of Variant Sequences: The epitopes presented in table 1 are based on B95.8 sequence of  
10     Epstein-Barr virus. We have examined field isolates of the virus from Papua New Guinea, Australia and Kenya and sequenced these at the sites of two CTL epitopes. These epitopes are AVLLHEESM and YPLHEQHGM. The results presented in Tables 1 and 2 and demonstrate that there is  
15     variation at the site of these epitopes in field isolates. At this stage, it is not known whether these variant sequences are CTL epitopes. If subsequent tests demonstrate that these represent active epitopes, then each could be included in a peptide-based vaccine.

**Table 2: Variation in the HLA B35-restricted EBNA4  
Epitope AVLLHEESM in Different Ethnic Groups**

Virus Isolate	Origin	Epitope Sequence
B95.8 JB LC DD MB GK AF6 AF7 AF19 RM AF1 LP AF3 AF5 AF13	Caucasian Caucasian Caucasian Caucasian Caucasian Kenyan Kenyan Kenyan Caucasian Kenyan Caucasian Kenyan Kenyan Kenyan	GCA GTT CTA CTT CAC GAA GAA TCC ATG A V L L H E E S M
JD RE PM H25 H19 H33 L5 L23 H36 H21 AF16 H7	Caucasian Caucasian Caucasian PNG* High PNG High PNG High PNG Low PNG Low PNG High PNG High Kenyan PNG High	ACA GTT CTA CTT CAC GAA GAA TCC ATG T V L L H E E S M
L42 H23 L43 L8 H26 H35	PNG Low PNG High PNG Low PNG Low PNG High PNG High	ACA GCT CTA CTT CAT GAA GAA TCC ATG T A L L H E E S M

5 \* Papua New Guinea

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## CLAIMS:-

1. A cytotoxic Epstein-Barr virus T-cell epitope, the epitope being selected from the group consisting of QAKWRLQTL, RYSIFFDY, HLAAQGMAY, YPLHEQHGM, SVRDRLARL,  
5 AVLLHEESM, VSFIEFVGW, FRKAQIQGL, PYLFWLAAI, TVFYNIPPMPL, PGDQLPGFSDGRACPV, VEITPYKPTW, and variants thereof.
2. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is QAKWRLQTL.
3. A cytotoxic Epstein-Barr virus T-cell epitope as  
10 claimed in claim 1 in which the epitope is RYSIFFDY.
4. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is HLAAQGMAY.
5. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is YPLHEQHGM.
- 15 6. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is SVRDRLARL.
7. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is AVLLHEESM.
8. A cytotoxic Epstein-Barr virus T-cell epitope as  
20 claimed in claim 1 in which the epitope is VSFIEFVGW.
9. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is FRKAQIQGL.
10. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is PYLFWLAAI.
- 25 11. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is TVFYNIPPMPL.
12. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is  
PGDQLPGFSDGRACPV.
- 30 13. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is VEITPYKPTW.
14. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is a variant of YPLHEQHGM, the variant being selected from the group  
35 consisting of YPLHKOHGM, YRLHEQHGM and YPLHEQRM.

15. A cytotoxic Epstein-Barr virus T-cell epitope as claimed in claim 1 in which the epitope is a variant of AVLLHEESM, the variant being selected from the group consisting of TVLLHEESM and TALLHEESM.

5 16. A composition for use in inducing CTL's in a subject, the composition comprising at least one cytotoxic Epstein-Barr virus T-cell epitope as claimed in any one of claims 1 to 15 in admixture with at least one pharmaceutical acceptable adjuvant, carrier, diluent or  
10 excipient.

17. A method of preparing a composition for use in inducing CTL's in a subject, the method comprising admixing at least one cytotoxic Epstein-Barr virus T-cell epitope as claimed in any one of claims 1 to 15 with at  
15 least one pharmaceutical acceptable adjuvant, carrier, diluent or excipient.

1/3

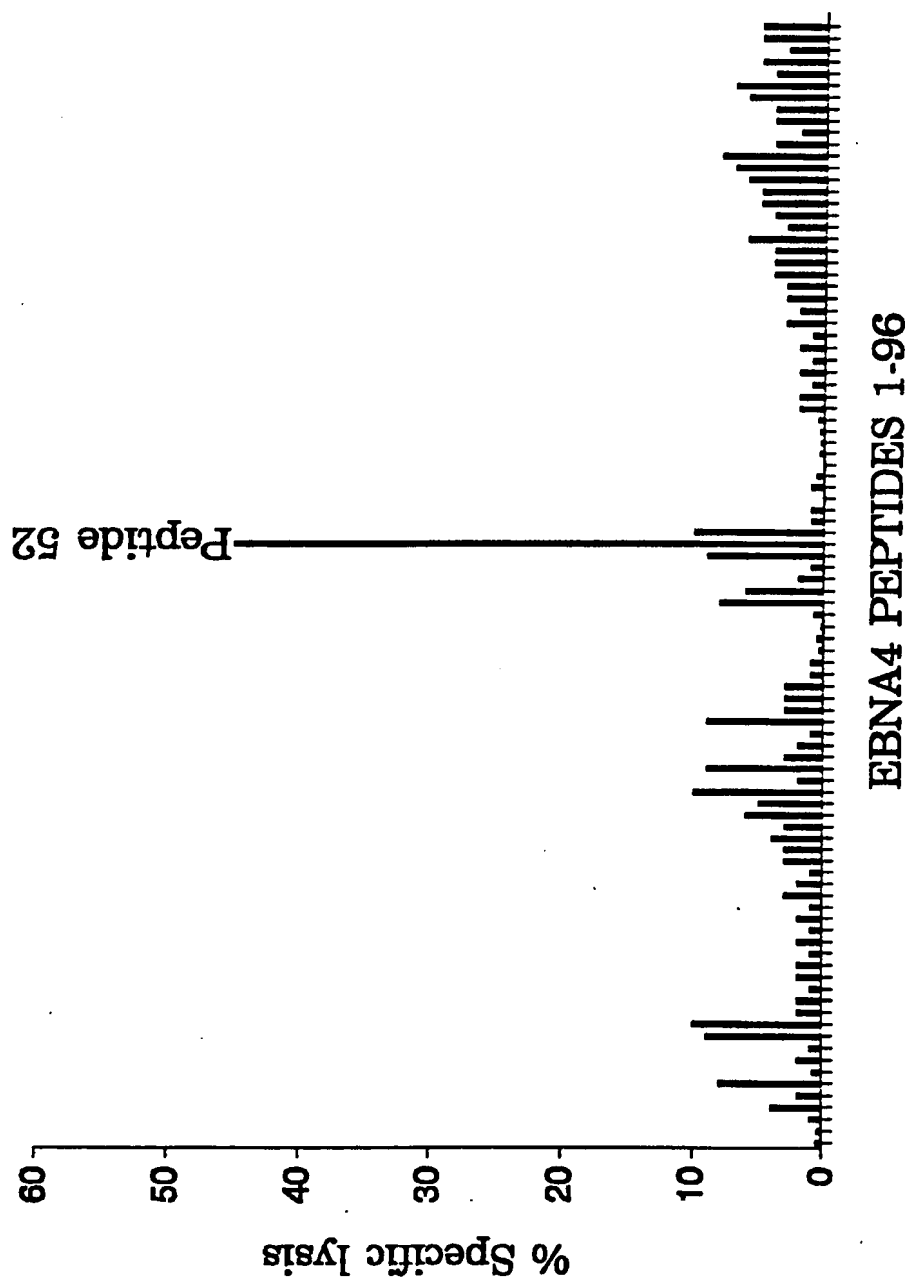
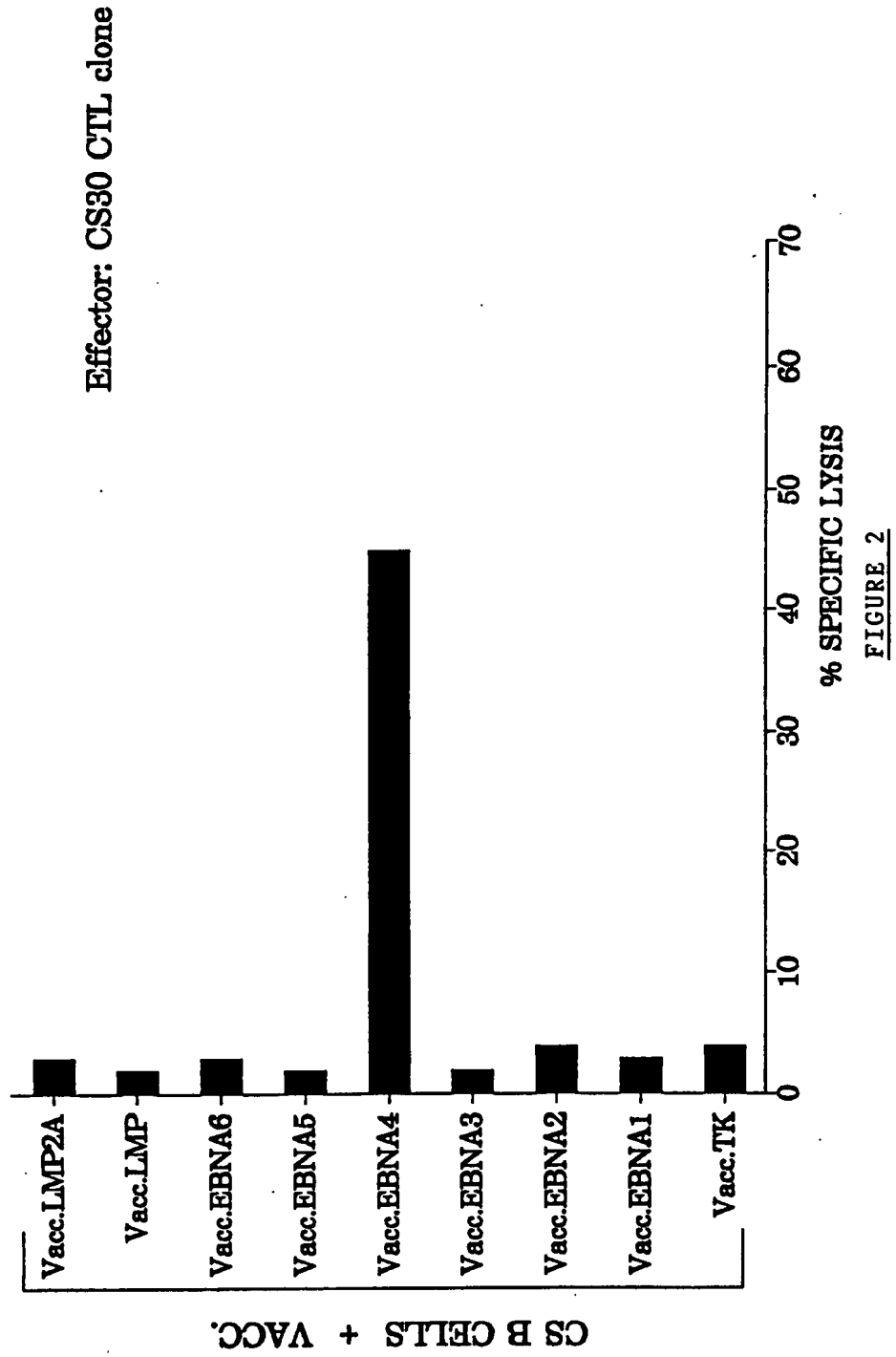


FIGURE 1

2/3



3/3

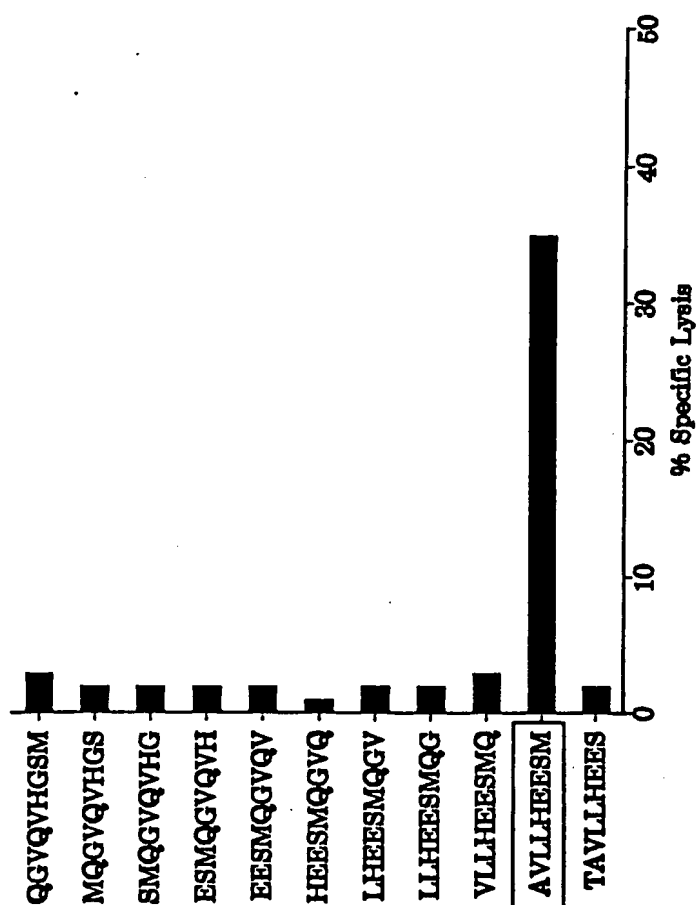


FIGURE 3

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/AU 95/00140

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int. Cl. <sup>6</sup> A61K 39/245, C07K 7/06, C07K 14/05  According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b>  Minimum documentation searched (classification system followed by classification symbols) IPC <sup>6</sup> A61K 39/245, C07K 14/05; CHEMICAL ABSTRACTS  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  Electronic data base consulted during the international search (name of data base, and where practicable, search terms used) DERWENT WPAT; Chemical Abstracts CASM; STN sequence search Keyword: Epstein () Barr				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
<b>Category*</b>	<b>Citation of document, with indication, where appropriate, of the relevant passages</b>	<b>Relevant to Claim No.</b>		
P,X	Burrows, S.R. et al. (1994) Five new cytotoxic T cell epitopes identified with Epstein-Barr virus nuclear antigen 3, volume 75, pages 2489-2493. See entire document.	1-17		
Y	Burrows, S.R. et al. (1992) Rapid visual assay of cytotoxic T-cell specificity utilizing synthetic peptide induced T-cell-T-cell killing, Immunology, volume 76, pages 174-175. See the third paragraph in particular.	1-17		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.         </div> <div style="width: 45%;"> <input checked="" type="checkbox"/> See patent family annex.         </div> </div>				
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 50%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> </td> </tr> </table>			<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>
<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>			
Date of the actual completion of the international search 9 June 1995		Date of mailing of the international search report 19 JUNE 1995 (19.06.95)		
Name and mailing address of the ISA/AU  AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA  Facsimile No. 06 2853929		Authorized officer  DAVID HENNESSY  Telephone No. (06) 2832255		

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 95/00140

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
Y	Khanna, R. et al. (1992) Localization of Epstein-Barr virus cytotoxic T-cell epitopes using recombinant vaccinia: Implications for vaccine development, J. Exp. Med., volume 176, pages 169-176, July 1992. See whole document.	1-17
Y	Burrows, S.R. et al. (1992) The specificity of recognition of a cytotoxic T lymphocyte epitope, Eur. J. Immunol., volume 22, pages 191-195. See whole document.	1-17
X,P	Lee, S.P. et al. (1995) Epstein-Barr virus isolates with the major HLA B35.01 restricted cytotoxic T lymphocyte epitope are prevalent in a highly B35.01-positive African population, Eur. J. Immunol., volume 25, pages 102-110. See the tables and figures in particular.	14-17
X	Khanna, R. et al. (1993) EBV peptide epitope sensitization restores human cytotoxic T cell recognition of Burkitt's lymphoma cells, The Journal of Immunology, volume 150, no. 11 pages 5154-5162, 1 June 1993.	1, 11
Y		2-10, 12-17
A	AU,A, 16480/92 (C.N.R.S. et al) published 30 September 1993.	

**INTERNATIONAL SEARCH REPORT**

International application No.

**PCT/AU 95/00140**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
AU,A, 16480/92	WO 93 19092
END OF ANNEX	